Identification of potato cyst nematode in Indonesia by polymerase chain reaction

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Abstract Polymerase chain reaction (PCR) with specific primers was used to amplify the ITS-rDNA regions of potato cyst nematodes (PCN) in soil samples from Indonesia. Amplified DNA fragments showed that a single species, *Globodera rostochiensis*, with fragment size 238 bp occurred in East Java, and West Java, while mixed populations of *G. rostochiensis* and *G. pallida* with fragment size 391 bp were only found in Central Java.

Keywords *Globodera rostochiensis* · *G. pallida*

Potato Cyst Nematode (PCN) is the most important plant parasitic nematode of potatoes (*Solanum tuberosum*). It has spread from Europe to tropical and subtropical regions of Asia, such as Pakistan, India, Philippines (Jatala and Bridge 1990). In March 2003, potato cyst nematodes were found in East Java, Indonesia (Indarti et al. 2004). In further investigations we have found the nematode in Central Java and West Java and now it is a damaging pathogen in potato plantations in Indonesia. To control PCN effectively, it is important to identify its species and the pathotypes. Traditionally, routine soil sampling and identification of PCN in Indonesia has been based on examination of morphological features. However, this is time-consuming and frequently unreliable because of morphological similarity of the two PCN species, *Globodera rostochiensis* and *G. pallida*.

Identification by molecular techniques, for example DNA finger printing, offers a more rapid and precise identification of PCN species. Here, we report studies conducted to identify Indonesian populations of PCN by polymerase chain reaction (PCR).

A soil survey was conducted at potato plantations in 24 regions of Java Island. A total of 40 soil samples were collected from these regions. Twenty samples were found to be infested with PCN in 11 regions (Table 1). Soil samples consisting of 100 cores of 15–20 g of soil were taken in a grid pattern throughout the plantations (EPPO 1998). Each 1,500–2,000 g composite sample was collected from soil down to a depth of approximately 100 mm, placed in a plastic bag and was labelled with the producer's name, field location, collection date, GPS coordinates, and sample number for laboratory identification. Samples were put into coolers for storage and transportation from the field and were submitted for processing to the Laboratory of Nematology, Department of Plant Protection, Faculty of Agriculture, Bogor Agricultural University, Bogor. Cysts were collected from 100 g soil sub-samples using a pair of sieves (840 and 250 mesh size) (Sheperd 1985) and then stored in Eppendorf tubes at room temperature.

DNA was extracted using the modified method described by Subbotin et al. (2001) and Fullaondo et al. (1999). Five cysts from each region were collected randomly into an Eppendorf tube filled with 150 µl lyses buffer (125 mM KC; 25 mM Tris-HCl, pH 8.0; 3.75 mM MgCl₂; 2.5 mM DTT; 1.125 % Tween 20 and 0.025 % gelatin) and 5 µl Proteinase K (600 µg/ml) (USB, UK) and were crushed using a plastic pistil. Following consecutive incubation periods at 65 °C for 1 h and 95 °C for 10 min, and centrifugation at 11,000 g for 10 min. DNA then was purified into chloroform-isoamyl alcohol (24:1) and precipitated with 3 M sodium acetate and absolute ethanol. DNA extract was resuspended into 20 µl sterile water and kept at –20 °C. Three primers used were as follows; specific primer for

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Table 1 Regions in Java Island Indonesia in which cysts of Potato Cyst Nematode (PCN) were collected from soil and codes of isolates used in this study

Regions of Indonesia		Populations of PCN	
Province	City and District	No. cysts/100 g soil	Isolate codes
East Java (location 1)	Tulung Rejo, Kota Batu	675	S1
East Java (location 2)	Tulung Rejo, Kota Batu	44	S2
East Java (location 3)	Tulung Rejo, Kota Batu	21	S3
Central Java	Pawuhan, Banjarnegara	400	S4
Central Java	Karangtengah, Banjarnegara	270	S5
Central Java	Patak Banteng, Wonosobo	2	S6
Central Java	Kepakisan, Banjarnegara	21	S7
West Java (location 3)	Sukamanah, Pengalengan	2	S8
West Java (location 4)	Sukamanah, Pengalengan	19	S9
West Java (location 5)	Sukamanah, Pengalengan	28	S10
West Java	Mekarwangi, Sindangkerta	17	S11

G. rostochiensis: ITS-1 (5'-TGT TGT ACG TGC CGT ACC TT-3'), *G. pallida*: ITS-1 (5'-GGT GAC TCG ACG ATT GCT GT-3'), and universal primer for PCN: 5.8S rDNA (5'-GCA GAA GGC TAG CGA TCT TC-3') (Mulholland et al. 1996). Twenty five ng DNA template from each region was transferred into 25 µl PCR mixture containing 50 mM Tris-HCl (pH 9.0), 50 mM KCl, 1.5 mM MgCl₂ so that the 2 is subscript, 0.1 % Triton X-100, 0.2 mM dNTP (New England Biolabs, Hitchin, UK), 50 ng primer and 0.5 unit Taq polymerase (New England Biolabs, Hitchin, UK). PCR products were analysed using 2 % agarose gel electrophoresis in 0.5× TBE buffer and stained with ethidium bromide (Maniatis et al. 1989).

The primers successfully amplified targeted DNA fragments for each PCN region. Paired primers of ITS-1 and 5.8S rDNA produced 238 bp DNA fragment for all regions (West Java, East Java, and Central Java) (Fig. 1),

Fig. 1 Amplified PCR products of cysts of potato cyst nematode collected in Indonesia using specific and universal *G. rostochiensis* primers (Mulholland et al. 1996). M, 100 bp ladder; 1–3 are isolates S1, S2 and S3 from East Java; 4–7 are isolates S4, S5, S6 and S7 from Central Java; 8–11 are isolates S8, S9, S10 and S11 from West Java

while ITS-1 and 5.8S rDNA resulted in a 391 bp DNA fragment only for the Central Java isolates (Fig. 2). According to Mulholland et al. (1996) the fragments of 238 bp and 391 bp are related to *G. rostochiensis* and *G. pallida*, respectively.

The results presented here give evidence of the occurrence of *G. rostochiensis* and *G. pallida* in Indonesia. Both species are present in Central Java (Wonosobo and Banjarnegara Districts), while in East Java and West Java only single species were found. This is the first report of identification with molecular characterisation of PCN in Indonesia. This method is not only highly effective as a species diagnostic tool but also a sensitive method which can be used for taxonomic and quarantine purposes.

Fig. 2 Amplified PCR products of cysts of potato cyst nematode collected in Indonesia using specific and universal *G. pallida* primers (Mulholland et al. 1996). M, 100 bp ladder; 1–4 are isolates S4, S5, S6 and S7 from Central Java

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